#12 4/30/01

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Re:	Patent Application for Kaster	**Dat :**	April 20, 2001
Serial No.:	09/100,516	**Art Unit:**	1638
Filed:	June 19, 1998	**Examiner:**	Dr. Gary Benzion
For:	Plants and Their Preparation	**Action:**	Information Disclosure with articles along with Form PTO-1449

To: The Commissioner of Patents and Trademarks, Washington, DC 20231

The documents identified on the attached for PTO-1449 have come to the attention of the undersigned in connection with the subject application. Copies of these documents are also attached, unless otherwise indicated below, and it is respectively requested that they be made of record in this proceeding. The identification of these documents is for the purpose of meeting Applicant's duty of disclosure under 37 C.F.R. 1.56 and is not intended to be an admission that any of these documents constitute prior art as to the invention disclosed in the subject application.

PCT PUBLICATION OF APPLICATION FOR PATENT COVER PAGE

WO9723634 A2 PUBLISHED JULY 3, 1997

TITLED: PRODUCTION OF TRUEBREEDING TRANSGENCIS SEEDS FROM PLANTS HETEROZYGOUS FOR TRANSGENE INSERTATIONS

THE US PATENT 6,057,496 FILED JUNE 22, 1998 AND ISSUED MAY 2, 2000

TITLED: TRUE BREEDING TRANSGENCIS FROM PLANTS HETEROZYGOUS FOR TRANSGENE INSERTATIONS

REMARKS

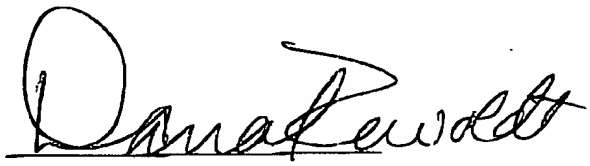
The above identified PCT publication is not considered to be prior art in light of the July 1997 publication date being within one year of the filing of the present application in the U.S. patent office. Additionally the filing of the present application was on June 19, 1998. The U.S. filing through PCT of the above identified US Patent 6,057,496 was on June 22, 1998. Therefore, it is believed that US Patent 6,057,496 is not prior art under U.S. law to the present invention. In spite of the fact that it is not prior art in the U.S. the applicant thought it best to provide it to the Examiner. If the Applicant is incorrect and this is prior art then the applicant submits as follows:

04/20/2001 GPAYNE 00000004 09100516

01 FC:126 180.00 OP

This publication teaches away from the use of the present method. Clearly, this publication focused on varietal plants that are not in hybrid production. Instead this publication teaches that this technology should be used in open pollinated varieties. In fact on page 24 submitted here in the Patent clearly teaches that use of herbicide on the pollen is only a minor component of the skewed segregation and that the dramatic effect is actually on the spraying of the ovule parent. And that the major component of the skewed segregation arises not from elimination of herbicide sensitive pollen but form the elimination of herbicide-sensitive ovules. Thus the reference does not suggest nor teach the preser t invention.

Respectfully submitted,



Dana Rewoldt, #33,762
Garst Seed Company
2369 330th Street, Box 500
Slater, IA 50244
(515) 685-5113

CERTIFICATE OF MAILING UNDER 37 C.F.R. 1.6

I hereby certify that the attached Information Disclosure and form 1449 and reference and the transmital and credit card fee payment voucher are being deposited with the United States Patent office by facsimile transmission to, on this 20th day of April, 2001.

703-308-4556

D Reinders

Diana Reinders

CERTIFICATE OF MAILING UNDER 37 C.F.R. 1.6

I hereby certify that the attached Information Disclosure and form 1449 and reference and the transmital and credit card fee payment voucher are being deposited with the United States Patent office by facsimile transmission to, on this 20th day of April, 2001. 703-308-4556

Diana Reinders

WO9723634A2:PRODUCTION OF TRUEBREEDING TRANSGENICS SEEDS FROM PLANTS HETEROZYGOUS FORTRANSGENE INSERTIONS

View Images (22 pages) | View Cart | View INPADOC only | Derwent Record...

Add to cart: PDF (~2090 KB) | TIFF | Fax | File History | More choices...

Country:	**WO** World Intellectual Property Organization (WIPO)
Kind:	**A2** Publ.OF the Int.Appl. without Int.Search REP.
Inventor(s):	**CONNER, Anthony, John** , 17A Dinton Street, Christchurch 8004, New Zealand
Applicant(s):	**NEW ZEALAND INSTITUTE FOR CROP & FOOD RESEARCH LIMITED**, Canterbury Agriculture and Science Centre, Ellesmere Road, Lincoln, Canterbury, New Zealand News, Profiles, Stocks and More about this company
Issued/Filed Dates:	**July 3, 1997** / Dec. 20, 1996
Application Number:	**WO1996NZ0000148**
IPC Class:	**C12N 15/82;** A01H 5/10; A01H 1/04;
ECLA Code:	**C12N15/82A8; A01H1/04; A01H1/06;**
Priority Number(s):	Dec. 21, 1995 **NZ1995000280742**
Legal Status:	Show legal status actions
Designated Countries:	AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, **European patent:** AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, **OAPI patent:** BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG, **ARIPO patent:** KE, LS, MW, SD, SZ, UG, **Eurasian patent:** AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

Abstract: The invention provides a method for biassing a crop plant which is heterozygous for a transgene towards the production of seeds which carry the transgene comprising the step of contacting a crop plant containing a gene construct comprising a transgene coding for resistance to a specific phytotoxin with said specific phytotoxin one or more times during the life of said plant. The method achieves this through selective inhibition of phytotoxinsensitive plant ovules.

Patent Available

United States Patent: 6,057,49

US PATENT & TRADEMARK OFFICE

PATENT FULL TEXT AND IMAGE DATABASE

United States Patent ***6,057,496***

Conner **May 2, 2000**

True breeding transgenics from plants heterozygous for transgene insertions

Abstract

The invention provides a method for biasing a crop plant which is heterozygous for a transgene towards the production of seeds which carry the transgene comprising the step of contacting the crop plant containing a gene construct comprising the transgene coding for resistance to a specific phytotoxin with the specific phytotoxin one or more times during the life of the crop plant. The method achieves this through selective inhibition of phytotoxin-sensitive plant ovules, embryos and pollen. The method has particular application to the production of open pollinated and synthetic varieties of crop plants, such as alfalfa synthetic varieties.

Inventors: **Conner; Anthony John** (Christchurch, NZ)

Assignee: **New Zealand Institute for Crop and Food Research Limited** (Cantebury, NZ)

Appl. No.: **100879**

Filed: **June 22, 1998**

Foreign Application Priority Data

Dec 21, 1995[NZ] 280742

Current U.S. Class: **800/300**; 435/69.1; 435/418; 435/419; 435/468; 800/260; 800/278; 800/290; 800/295; 800/298; 800/306; 800/320; 800/323

Intern'l Class: C12N 015/82; A01H 001/00; A01H 005/00; A01H 005/10

Field of Search: 435/69.1,468,418,419 536/23.6 800/260,278,298,306,323,320,300,290,295

References Cited [Referenced By]

U.S. Patent Documents

5254801	Oct., 1993	Dotson et al.	800/278.
5278057	Jan., 1994	Jorgensen	435/468.
5426041	Jun., 1995	Fabijanski et al.	435/468.
5633441	May., 1997	De Greef et al.	800/278.
5652354	Jul., 1997	Mariana et al.	536/24.

Other References

Conner and Christey, Biocontrol Science and Technology, 4:463-473 (1994).
Bayley et al., "Engineering 2,4-D Resistance Into Cotton," Theor Appl Genet, 83:645-649 (1992).
Conner et al., "Brassica Napus Mutants With Increased Chlorsulfuron Resistance," Proc. 47th New Zealand Plant Protection Conf., 173-177 (1994).
Guerineau et al., "Sulfonamide Resistance Gene for Plant Transformation," Plant Molecular Biology, 15:127-136 (1990).
McKersie, B.D. and S.R. Bowley, "Somatic Embryogenesis: Forage Improvement using Synthetic Seeds and Plant Transformation," In Molecular and Cellular Technologies for Forage Improvement, Proceedings of a symposium sponsored by Divisions C-1, C-6, and C-7 of the Crop Science Society of America in Indianapolis, IN, Nov. 6, 1996, E.C. Brummer et al. (editors), CSSA Special Publication Number 26:117-134 (1998).
Potrykus, I. Ann. Rev. Plant Physiol. Mol. Biol., vol. 42, pp. 205-225, 1991.
Lyon et al, Plant Mol. Biol., vol. 13 pp. 533-540, 1989.
Li et al, Plant Physiol., vol. 100, pp. 662-668, 1992.
D'Ovidio, Plant Mol. Biol., vol. 22, pp. 1173-1176, 1993.
McKersie et al, Plant Physiol., vol. 103, pp. 1155-1163, 1993.
Weeks et al, Plant Physiol., vol. 102, pp. 1077-1084, 1993.
Odell et al, Mol. Gen. Genet., vol. 223, pp. 369-378, 1990.
White et al, Plant Mol. Biol., vol. 8, pp. 461-469, 1987.

Primary Examiner: Fox; David T.
Assistant Examiner: Mehta; Ashwin
Attorney, Agent or Firm: Morgan, Lewis & Bockius LLP

Parent Case Text

CROSS REFERENCES TO RELATED APPLICATIONS

This application claims priority to PCT/NZ96/00148, filed on Dec. 20, 1996, and New Zealand Patent Application No. 280742, filed on Dec. 21, 1995, both applications which are hereby incorporated by reference in their entirety.

Claims

What is claimed is:

1. A method for biasing a crop plant which is heterozygous for a transgene coding for resistance to a translocatable herbicide towards the production of seeds which carry the transgene comprising contacting the crop plant containing a gene construct comprising the transgene with the translocatable herbicide one or more times during the life of the crop plant, wherein the crop plant is part of an open-pollinated population or a synthetic variety, and subsequently collecting the seed produced by the crop plant and confirming the presence of the transgene.

2. A method of selectively inhibiting herbicide-sensitive plant ovules, embryos and/or pollen in order to bias a crop plant which is heterozygous for a transgene coding for resistance to a translocatable herbicide towards the production of seeds which carry the transgene comprising contacting the crop plant containing a gene construct comprising the transgene with the

translocatable herbicide one or more times during the life of the crop plant, wherein the crop plant is part of an open-pollinated population or a synthetic variety, and subsequently collecting the seed produced by the crop plant and confirming the presence of the transgene.

3. A method of selectively inhibiting herbicide-sensitive plant ovules in order to bias a crop plant which is heterozygous for a transgene coding for resistance to a translocatable herbicide towards the production of seeds which carry the transgene comprising contacting the crop plant containing a gene construct comprising the transgene with the translocatable herbicide one or more times during the life of the crop plant, wherein the crop plant is part of an open-pollinated population or a synthetic variety, and subsequently collecting the seed produced by the crop plant and confirming the presence of the transgene.

4. A method of selectively aborting herbicide-sensitive plant embryos in order to bias a crop plant which is heterozygous for a transgene coding for resistance to a translocatable herbicide towards the production of seeds which carry the transgene comprising contacting the crop plant containing a gene construct comprising the transgene with the translocatable herbicide one or more times during the life of the crop plant, wherein the crop plant is part of an open-pollinated population or a synthetic variety, and subsequently collecting the seed produced by the crop plant and confirming the presence of the transgene.

5. A method of selectively inhibiting herbicide-sensitive pollen in order to bias a crop plant which is heterozygous for a transgene coding for resistance to a translocatable herbicide towards the production of seeds which carry the transgene comprising contacting the crop plant containing a gene construct comprising the transgene with the translocatable herbicide one or more times during the life of the crop plant, wherein the crop plant is part of an open-pollinated population or a synthetic variety, and subsequently collecting the seed produced by the crop plant and confirming the presence of the transgene.

6. The method of claim 1 wherein the translocatable herbicide is contacted with said crop plant during the vegetative or reproductive growth phase of the crop plant.

7. The method of claim 1 wherein the crop plant is alfalfa or clover.

8. The method of claim 1 wherein the gene construct consists of a transgene coding for resistance to a translocatable herbicide operably linked to one or more additional transgene(s), such that the crop plant is biased towards the production of seeds which carry all of the transgenes.

9. The method of claim 1 wherein the translocatable herbicide is chlorsulfuron.

10. The method of claim 1 wherein the crop plant is a forage crop plant, a tree, a vegetable or a flowering plant.

11. The method as claimed in claim 10 wherein the forage crop plant is a forage brassica plant, a forage legume plant or a grass plant.

12. The method of claim 1 which includes the preliminary step of introducing the gene construct into the crop plant or into the seed from which the crop plant is grown.

13. The method as claimed in claim 12 wherein the gene construct consists of a transgene coding for resistance to a translocatable herbicide and wherein the construct is introduced into the genome of the crop plant at an integration site immediately adjacent a desirable homologous gene, such that when the crop plant is contacted with said translocatable herbicide the crop plant is biased towards the production of seeds which carry both the transgene and the homologous gene.

14. A method producing a transgenic synthetic alfalfa variety comprising the steps of:

1) producing a transgenic alfalfa plant, wherein the transgenic alfalfa plant is heterozygous for an introduced gene construct which comprises a heterologous gene coding for translocatable herbicide resistance operably linked to a heterologous gene of interest;

2) using the transgenic alfalfa plant produced by step 1) as a parent and crossing the transgenic alfalfa plant to one or more alfalfa plants which do not contain the translocatable herbicide resistance gene operably linked to the heterologous gene of interest;

3) applying the translocatable herbicide one or more times during the life cycle of the plants in step 2);

4) harvesting and bulking the resultant seed to produce a new synthetic alfalfa variety which contains the translocatable herbicide resistance gene operably linked to the gene of interest, and

5) confirming the presence of the translocatable herbicide resistance gene.

15. The method of claim 14 further comprising the following steps:

6) intermating the plants grown from the bulked seed obtained in step 4);

7) applying the translocatable herbicide one or more times during the life cycle of the plants in step 6); and,

8) harvesting and bulking the resultant seed, wherein such seed contains the translocatable herbicide resistance gene operably linked to the gene of interest.

16. The method of claim 1 wherein the open-pollinated population or synthetic variety is part of a mixture of two or more different crop plants.

17. The method of claim 16 wherein the two or more different crop plants are selected from the group consisting of forage brassica plants, forage legume plants and grass plants.

Description

FIELD OF THE INVENTION

The present invention pertains, in general, to methods for producing transgenic seeds. In particular, it relates to methods for ensuring that crop plants which are heterozygous for the presence of a transgene produce seeds which always carry the transgene.

BACKGROUND OF THE INVENTION

All publications and patent applications herein are incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

A major problem associated for the commercialization of transgenic cultivars in highly heterozygous crops is the segregation of transgenes during seed production (Conner and Christey Biocontrol Science and Technology 4:463-473 (1994)). In order to develop a cultivar involving crosses between heterozygous individuals (e.g.: asparagus, forage brassicas, pasture species, forest trees, etc.), it will be necessary to intermate individuals heterozygous for transgenes. In

many open pollinated or synthetic cultivars this will usually involve the intermating of several transgenic lines independently derived for different individual plants. This will involve the parents of synthetic cultivars, or a sufficient number of different individuals to maintain an effective population size to avoid inbreeding depression/genetic drift within the population. The transgenic individuals utilized in producing transgenic synthetic populations may arise from single event transformations of a single plant. When this is the case, the transgene could be introduced into a synthetic population, such as an alfalfa synthetic population, by making multiple crosses of the individual transgenic alfalfa plant with a number of different non-transgenic alfalfa plants from one or more alfalfa lines. Alternatively, since the transgenic individuals to be intermated may be derived from independently derived transformed plants, the transgenes may be located at different loci. The resulting intermated progeny will therefore be segregating at all the loci and the transgenic traits will have a "quantitative basis" (Conner and Christey, supra). As discussed immediately below, the prior art has failed to address the segregation and consequent loss of transgenes in open pollinated and synthetic populations.

U.S. Pat. No. 5,254,801 discloses methods whereby plant cells and whole plants can be genetically modified so as to selectively induce cellular lethality using heterologous dominant, conditionally lethal genes in combination with selected protoxin compounds. The methods are for inducing male sterility for the hybrid seed production, including alfalfa, canola, and oil seed rape. This patent fails to disclose a method of producing heterologous plants which utilizes a transgene coding for resistance to a specific phytotoxin.

U.S. Pat. No. 5,278,057 describes a method of producing plants with a marker closely linked to a target locus, in particular a nuclear male sterile target locus. The method involves transformation of a group of plants in order to introduce a marker into each plant, and isolation of a plant with the marker closely linked to a target locus. The markers include visible markers and dominant conditional lethal markers (e.g., antibiotic resistance or herbicide resistance). The method is of particular use for hybrid seed production of any crop plant where the target locus is a nuclear male sterile locus, including rapeseed, alfalfa, clover, cole crops or Brassica oleracea.

U.S. Pat. No. 5,426,041 discloses a method for seed preparation which comprises:

a) crossing a male sterile plant and a second plant which is male fertile,

b) obtaining seed of said male sterile plant, wherein the seed has integrated into its genome:

1) a first recombinant DNA molecule having a first DNA sequence which encodes a first gene product and a first promoter which is capable of regulating the expression of said first DNA sequence; and,

2) a second recombinant DNA molecule which contains a second DNA sequence which encodes a second gene product and a second promoter which is capable of regulating the expression of said second DNA sequence.

The first and second gene products cooperate to selectively interfere with the function and/or development of cells of a plant that are critical to pollen formation and/or function of a plant grown from said seed, such that any plant grown from the seed is substantially male sterile.

More specifically, the '041 patent further teaches a procedure to produce hybrid seed which includes using an IamS/IamH genetic system. The procedure can include linking the IamH gene to a gene for herbicide resistance so that the herbicide can be used for the roguing of the plant line A1; and, that herbicide application takes place after flowering and will kill the A1 so that only seed that has the genotype A1/A2 is produced. The A1/A2 seed is substantially 100% male sterile and can be pollinated with a male fertile line leading to commercial hybrid seed.

U.S. Pat. No. 5,633,441 is directed to plants comprising female-sterility DNA encoding a protein or polypeptide such as barnase which, when produced in the cells of the plant, kills or significantly disturbs the metabolism, functioning or development of the cells. The foreign DNA also comprises a first promoter which directs expression of the female-sterility DNA selectively in style cells, stigma cells or style and stigma cells of the female reproductive organs of the plants. The first promoter does not direct detectable expression of the female sterility DNA in the ovule or in other parts of the plant so that the plant remains male-fertile. The female-sterility DNA is in the same transcriptional unit as and under the control of the first promoter. More specifically, the '441 patent discloses a foreign chimaeric DNA sequence that comprises the female-sterility DNA and a first promoter and that can also comprise a marker DNA and a second promoter. Preferred markers include herbicide tolerance or resistance genes.

The '441 patent further discloses a process for producing hybrid seeds, which grow into hybrid plants, by crossing: 1) the female-sterile plant of this invention which may include, in its nuclear genome, the marker DNA, preferably encoding a protein conferring a resistance to a herbicide on the plant; and 2) a female-fertile plant without the marker DNA in its genome.

U.S. Patent No.5,652,354 relates to promoters from endogenous genes of plants, wherein said promoters direct gene expression selectively in stamen cells of said plant, particularly in tapetum cells of said plant. The promoters may be used to transform a plant with a foreign DNA sequence encoding a product which selectively disrupts the metabolism, functioning, and/or development of stamen cells of the plant. The male-sterility DNA and its associated promoter are exemplified as being foreign DNA sequences. Preferred marker DNAs are those which inhibit or neutralize the action of herbicides.

None of these patents discloses a method of producing an open-pollinated population or synthetic variety whereby a transgene is maintained at sufficiently useful levels during subsequent generations of inter- and intra-crossing of the parental lines which made up the original population or variety.

It would be highly desirable to have a method to prevent the formation of, or eliminate, the individual seeds that do not carry a transgene. If this could be achieved, all the seeds in subsequent generations would carry a transgene, without interfering with the highly heterozygous genetic background of the cultivar. It would also offer a more convenient strategy for introgression of transgenes into open pollinated crop cultivars. A single transgenic individual could be intermated to many other individuals, with the high proportion of non-transgenic progeny being prevented from developing in seed production blocks prior to or during flowering and seed development.

Thus, the object of this invention to provide methods for producing segregating populations in which one or more transgenes are maintained in a large enough percentage of the plants so that the beneficial effect of the transgenes are realized.

SUMMARY OF THE INVENTION

In one aspect, the present invention can be said to broadly consist in a method for biasing a crop plant which is heterozygous for a transgene towards the production of seeds which carry the transgene comprising the step of contacting a crop plant containing a gene construct comprising a transgene coding for resistance to a specific phytotoxin with said specific phytotoxin one or more times during the life of said plant.

In still another aspect the invention provides a method of selectively inhibiting phytotoxin-sensitive plant ovules, embryos and/or pollen in order to bias a crop plant which is heterozygous

for a transgene towards the production of seeds which carry the transgene comprising the step of contacting a crop plant containing a gene construct comprising a transgene coding for resistance to a specific phytotoxin with said specific phytotoxin one or more times during the life of said plant.

In yet another aspect, the invention provides a method of selectively inhibiting phytotoxin-sensitive plant ovules in order to bias a crop plant which is heterozygous for a transgene towards the production of seeds which carry the transgene comprising the step of contacting a crop plant containing a gene construct comprising a transgene coding for resistance to a specific phytotoxin with said specific phytotoxin one or more times during the life of said plant.

In a further aspect, the invention provides a method of selectively aborting phytotoxin-sensitive plant embryos in order to bias a crop plant which is heterozygous for a transgene towards the production of seeds which carry the transgene comprising the step of contacting a gene construct comprising a transgene coding for resistance to a specific phytotoxin with said specific phytotoxin one or more times during the life of said plant.

In yet a further aspect, the invention provides a method of selectively inhibiting phytotoxin-sensitive pollen in order to bias a crop plant which is heterozygous for a transgene towards the production of seeds which carry the transgene comprising the step of contacting a crop plant containing a gene construct comprising a transgene coding for resistance to a specific phytotoxin with said specific phytotoxin one or more times during the life of said plant.

In addition to the phytotoxin resistance gene, the gene construct may also contain additional liked transgene(s) which are co-transferred to the transgenic plant.

Conveniently, the method includes the preliminary step of introducing said gene construct into said plant or into the seed from which said plant is grown.

In preferred embodiments, the method includes the subsequent step of collecting the seed produced by the plant, and confirming the presence of the gene construct.

The phytotoxin which is applied to the plant can be an antibiotic or a herbicide. It is however presently preferred that the phytotoxin be a herbicide. It is further preferred that the herbicide be one which translocates throughout the plant upon application.

In a further embodiment, the invention provides seeds carrying a gene construct produced by the method defined above.

Although the present invention is broadly as defined above, it will be appreciated by those person skilled in the art that it is not limited thereto and that it further includes the embodiments which are described below.

Further objects and advantages of the present invention will be clear from the description that follows.

DETAILED DESCRIPTION OF THE INVENTION

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are described.